

WHAT IS CLAIMED IS:

1                   1.       A method of regulating the replication of a DNA molecule, the  
2 method comprising,  
3                   introducing into a eukaryotic cell,  
4                   a) a replication cassette comprising an origin of replication; and  
5                   b) a replication system comprising  
6                   i)       a polynucleotide encoding a polypeptide with RNA  
7 polymerase activity;  
8                   ii)     a polynucleotide encoding a polypeptide with DNA  
9 polymerase activity;  
10                  iii)    a polynucleotide encoding a polypeptide with DNA  
11 helicase activity and;  
12                  iv)    a polynucleotide encoding a polypeptide with DNA  
13 primase activity;  
14                   wherein the polynucleotide encoding each polypeptide is operably  
15 linked to a eukaryotic replication promoter, thereby initiating replication of the replication  
16 cassette independent from chromosomal DNA replication.

1                   2.       The method of claim 1, wherein the replication system comprises a  
2 polynucleotide encoding each of the following polypeptides: T7 RNA polymerase, T7  
3 gene 4 protein, T7 DNA polymerase and TrxA.

1                   3.       The method of claim 2, wherein the eukaryotic cell is a plant cell.

1                   4.       The method of claim 2, wherein the eukaryotic cell is a mammalian  
2 cell.

1                   5.       The method of claim 2, wherein the origin of replication is a T7  
2 bacteriophage origin of replication.

1                   6.       The method of claim 2, wherein the replication cassette comprises  
2 a T7 promoter.

1                   7.       The method of claim 2, wherein the replication cassette comprises  
2 an expression cassette.

1                   8.       The method of claim 2, wherein the expression cassette comprises  
2 a polynucleotide operably linked to an expression promoter in an antisense orientation.

1                   9.       The method of claim 2, wherein the expression cassette comprises  
2 a polynucleotide operably linked to an expression promoter in a sense orientation.

1                   10.      The method of claim 2, wherein the replication cassette comprises  
2 at least 200 base pairs of DNA that is at least 70% identical to chromosomal DNA in the  
3 eukaryotic cell.

1                   11.      The method of claim 10, wherein the replication cassette comprises  
2 at least 200 base pairs of DNA that is identical to chromosomal DNA in the eukaryotic  
3 cell.

1                   12.      The method of claim 2, wherein the replication cassette comprises  
2 a recombination sequence.

1                   13.      The method of claim 12, wherein the recombination sequence is a  
2 *lox* sequence.

1                   14.      The method of claim 2, wherein the replication system  
2 polynucleotide(s) further encode a sequence-specific recombinase operably linked to a  
3 promoter.

1                   15.      The method of claim 14, wherein the sequence-specific  
2 recombinase is the Cre recombinase.

1                   16.      The method of claim 2, wherein the eukaryotic replication  
2 promoter is tissue-specific.

1                   17.      The method of claim 2, wherein the eukaryotic replication  
2 promoter is constitutive.

1                   18.      The method of claim 2, wherein the eukaryotic replication  
2 promoter is meiosis-specific.

1                   19.      The method of claim 2, wherein the eukaryotic replication  
2 promoter is inducible.

- 1                   20.     The method of claim 2, wherein at least one of the replication  
2     system polynucleotide(s) encoding T7 RNA polymerase, T7 gene 4 protein, T7 DNA  
3     polymerase and TrxA encode a nuclear localization signal.
- 1                   21.     The method of claim 20, wherein all of the replication system  
2     polynucleotide(s) encoding T7 RNA polymerase, T7 gene 4 protein, T7 DNA polymerase  
3     and TrxA encode a nuclear localization signal.
- 1                   22.     The method of claim 2, wherein the number of copies of the  
2     replication cassette is increased.
- 1                   23.     A eukaryotic organism comprising a polynucleotide encoding each  
2     of the following polypeptides: T7 RNA polymerase, T7 gene 4 protein, T7 DNA  
3     polymerase and TrxA, wherein the polynucleotide encoding each polypeptide is operably  
4     linked to a eukaryotic replication promoter.
- 1                   24.     The eukaryotic organism of claim 23, wherein the organism is a  
2     plant.
- 1                   25.     The plant of claim 24, further comprising a replication cassette  
2     comprising a bacteriophage T7 origin of replication.
- 1                   26.     The plant of claim 24, wherein the replication cassette comprises  
2     an expression cassette.
- 1                   27.     The plant of claim 24, wherein the expression cassette comprises a  
2     polynucleotide operably linked to an expression promoter in an antisense orientation.
- 1                   28.     The plant of claim 24, wherein the expression cassette comprises a  
2     polynucleotide operably linked to an expression promoter in a sense orientation.
- 1                   29.     The plant of claim 24, wherein the replication cassette is episomal.
- 1                   30.     The plant of claim 29, wherein the replication cassette is a plasmid.
- 1                   31.     The plant of claim 24, wherein the replication cassette is integrated  
2     into a eukaryotic chromosome.

1                    32.     The plant of claim 24, wherein the replication cassette comprises at  
2     least 200 base pairs of DNA that is at least 70% identical to chromosomal DNA in the  
3     plant cell.

1                    33.     The plant of claim 32, wherein the replication cassette comprises at  
2     least 200 base pairs of DNA that is substantially identical to chromosomal DNA in the  
3     plant cell.

1                    34.     The plant of claim 24, wherein the replication cassette comprises a  
2     recombination sequence.

1                    35.     The plant of claim 34, wherein the recombination sequence is a *lox*  
2     sequence.

1                    36.     The plant of claim 24, further comprising a polynucleotide  
2     encoding a sequence-specific recombinase operably linked to a promoter.

1                    37.     The plant of claim 36, wherein the sequence-specific recombinase  
2     is the Cre recombinase.

1                    38.     The plant of claim 24, wherein the eukaryotic replication promoter  
2     is tissue-specific.

1                    39.     The plant of claim 24, wherein the eukaryotic replication promoter  
2     is constitutive.

1                    40.     The plant of claim 24, wherein the eukaryotic replication promoter  
2     is meiosis-specific.

1                    41.     The plant of claim 24, wherein the eukaryotic replication promoter  
2     is inducible.

1                    42.     The plant of claim 24, wherein the polynucleotide(s) encoding T7  
2     RNA polymerase, T7 gene 4 protein, T7 DNA polymerase and TrxA each encode a  
3     nuclear localization signal.

1                    43.     A replication system comprising a polynucleotide encoding each of  
2     the following polypeptides: T7 RNA polymerase, T7 gene 4 protein, T7 DNA polymerase

3 and TrxA, wherein the polynucleotide encoding each polypeptide is operably linked to a  
4 eukaryotic replication promoter.

1 44. The replication system of claim 43, further comprising a  
2 polynucleotide encoding a sequence-specific recombinase.

1 45. The replication system of claim 44, wherein the sequence-specific  
2 recombinase is the Cre recombinase.

1 46. A polynucleotide, comprising  
2 a bacteriophage T7 origin of replication;  
3 a recombination sequence; and  
4 an expression cassette comprising a eukaryotic replication promoter.

1 47. The polynucleotide of claim 46, wherein the recombination  
2 sequence is a *lox* sequence.

1 48. The polynucleotide of claim 46, wherein the polynucleotide  
2 comprises a T7 promoter.